***Beutenbergia cavernae* gen. nov., sp. nov., an L-lysine-containing actinomycete isolated from a cave**

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Two aerobic, Gram-positive bacteria, strains HKI 0122^T and HKI 0132, were isolated from a cave. Cells are not acid-fast, non-motile, non-spore-forming and exhibit a rod-coccus growth cycle. The cell wall peptidoglycan contains lysine in position 3 of the peptide subunit and an interpeptide bridge of L-Lys ← L-Glu. The major menaquinone is MK-8(H₄), 13-methyl and 12-methyl tetradecanoic acids are the predominating fatty acids. The polar lipids consist of phosphatidylinositol, diphosphatidylglycerol and three unknown phospholipids. Mycolic acids are absent. The DNA base composition is 71 mol% G+C. Phylogenetic analysis revealed that strain HKI 0122^T forms a novel taxon among the families and unassigned genera of the suborder *Micrococcineae*, within the order *Actinomycetales*. On the basis of the genotypic, chemotaxonomic, morphological and physiological characteristics of these two isolates it is proposed to assign strains HKI 0122^T and HKI 0132 to a new genus and species for which the name *Beutenbergia cavernae* gen. nov., sp. nov. is proposed. The type strain is HKI 0122^T (= DSM 12333^T).

Keywords: *Beutenbergia cavernae* gen. nov., sp. nov., L-lysine-containing actinomycete

INTRODUCTION

The microbial diversity of as yet poorly studied biotopes is being increasingly explored by molecular detection methods and classical isolation techniques (Eppard *et al.*, 1996; Rheims *et al.*, 1996, 1998; Rainey, 1998; Waite *et al.*, 1998). While molecular methods are valuable tools in characterizing the microflora, isolation and culturing are required for describing the microbial diversity, especially in cases of novel taxa (Palleroni, 1997). Isolation procedures performed recently in karstic caves of northern Spain and southern Italy (Groth & Saiz-Jimenez, 1999; Groth *et al.*, 1999) revealed the occurrence of many different genera of actinomycetes. Applying similar isolation methods to a soil sample collected in the Reed Flute Cave near Guilin, Guangxi (China) two strains were found that were characterized by the common occurrence of the two chemotaxonomic properties, i.e. lysine as diagnostic diamino acid in the peptidoglycan and MK-

8(H₄) as the main menaquinone. Within the class *Actinobacteria* (Stackebrandt *et al.*, 1997) this combination of taxonomic markers has so far only been found in members of the genera *Bogoriella* (Groth *et al.*, 1997a) and *Demetria* (Groth *et al.*, 1997b). Similarity values of 16S rDNA sequences obtained for the type strains of these two genera, for other representatives of members of the order *Actinomycetales* and for the two cave isolates, showed the latter to be neither related to the genera *Bogoriella* or *Demetria* nor to any other genus of the order *Actinomycetales*. This distinct phylogenetic position was supported by chemotaxonomic, physiological and morphological characteristics.

On the basis of our results we concluded that both strains should be classified in a new genus and species for which the name *Beutenbergia cavernae* is proposed. The type strain is HKI 0122^T (DSM 12333^T).

METHODS

Bacterial strains and cultural conditions. Strains HKI 0122^T and HKI 0132 were isolated from a soil sample collected in a cleft between the rocks of the Reed Flute Cave near Guilin,

The EMBL accession number for the 16S rDNA sequence of HKI 0122^T is Y18378.

Fig. 1. 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances (De Soete, 1983) showing the phylogenetic position of strain HKI 0122^T next to members of the family *Intrasporangiaceae* and of other families of the suborder *Micrococccineae* (Stackebrandt *et al.*, 1997). Numbers at branching points refer to bootstrap values (500 trees resampled). Scale bar, 2 inferred nucleotide substitutions per 100 nucleotides.

Guangxi (China). One gram of soil was suspended in 10 ml sterile phosphate buffer (pH 7.2) and thoroughly mixed by shaking. Soil particles were allowed to sediment, the liquid phase was serially diluted and spread onto the surface of casein mineral medium (Altenburger *et al.*, 1996) which contains per litre: 0.6 g K_2HPO_4 , 0.5 g $Na_2HPO_4 \cdot 2H_2O$, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.1 g $MgCl_2 \cdot 7H_2O$, 0.2 g KNO_3 , 0.01 g $FeCl_3 \cdot 6H_2O$, 0.8 g casein and 0.4 g yeast extract (pH 7.0) and peptone/yeast extract/brain-heart infusion medium (Yokota *et al.*, 1993) in the case of HKI 0132. Solidified rich (R) medium (Yamada & Komagata, 1972) was used for general laboratory cultivation including morphological and physiological studies unless otherwise indicated. Biomass for biochemical analyses was prepared by cultivating the strains in liquid R medium or Bacto tryptic soy broth (Difco) and harvesting the cells by centrifugation.

Morphological and physiological characteristics. Cell morphology was examined by phase-contrast microscopy (Olympus BH-2 microscope). Motility was studied by the hanging drop technique. Cell dimensions were measured microscopically [Olympus BX 50, equipped with the image analysing software VIS 5504 H (Jentech)]. Gram staining and acid-fast staining were performed as described previously (Groth *et al.*, 1997b). For scanning electron microscopy, a 72-h-old culture grown on a R agar plate was suspended in phosphate buffer (pH 7.2). Preparation of cells was as described by Groth *et al.* (1997b). Cell morphology was studied with a field emission scanning electron microscope (LED 1530) with a working distance of 3 mm. Colony morphology was studied with a stereo microscope (Olympus SZ 11). Growth dependence on different incubation temperatures, increasing concentrations of NaCl in the culture medium and on the supply with oxygen as well as the utilization of various organic compounds and selected

enzyme activities were studied as described by Groth *et al.* (1997b). The susceptibility to antibiotics was tested with antibiotic discs (Oxoid) on R agar plates seeded with suspensions of HKI 0122^T and HKI 0132, respectively.

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer & Kandler (1972). The amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). The amino terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (1985). Whole-cell sugars were determined as alditol acetates by GC as described by Groth *et al.* (1996). The molar ratios of amino acids were determined by GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987). Analysis of enantiomers of cell wall amino acids was performed by GC of *N*-pentafluoropropionyl amino acid isopropyl esters (Frank *et al.*, 1980) on a L-Chirasil-Val column (25 m × 0.25 mm i.d.; Macherey-Nagel) using helium as carrier gas at a linear velocity of 200 mm s⁻¹ and at a temperature programmed from 80 to 190 °C at a rate of 2 °C min⁻¹. Instruments used for GC and GC-MS were as specified previously (Groth *et al.*, 1996). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida & Aida (1984).

Lipid analysis. Cellular fatty acid methyl esters obtained from cells grown in Bacto tryptic soy broth at 28 °C by the method of Stead *et al.* (1992) were separated by GC (Groth *et al.*, 1996) and identified as described previously (Schumann *et al.*, 1997). Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997a). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-

dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Absence of mycolic acids was demonstrated by TLC (Minnikin *et al.*, 1975).

DNA base composition. DNA was isolated and its G+C content determined by HPLC of deoxyribonucleosides as described by Groth *et al.* (1996).

16S rDNA sequence determination and phylogenetic analysis. Preparation of genomic DNA from strains HKI 0122^T and HKI 0132 was done as described previously (Rainey *et al.*, 1996). Sequences were aligned manually with sequences published previously. These were stored in the DSMZ 16S rDNA database consisting of several thousand 16S rDNA sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 1997) and EMBL. Similarity values were transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of De Soete (Maidak *et al.*, 1997) and the neighbour-joining and the maximum-likelihood methods contained in the PHYLIP package (Felsenstein, 1993) were used in the construction of phylogenetic dendrograms. Bootstrap values were determined according to Felsenstein (1993). All analyses were done on a SUN SparcII workstation.

Nucleotide accession numbers. The accession numbers of the reference strains used in the phylogenetic analysis are shown in Fig. 1.

RESULTS

Morphological and physiological characteristics

Colonies of strain HKI 0122^T grown on R agar plates were circular, glistening, convex, smooth, opaque with an entire margin. They varied in colour from cream to bright yellow. The diameters of the colonies were in the range 0.7–1.8 mm. During growth in complex media a rod-coccus growth cycle occurred. Young cultures (about 24 h) were composed almost entirely of irregular rods arranged in palisades, clusters or in pairs at an angle to give V-forms (Fig. 2a). In stationary-phase cultures coccoid cells dominated occurring singly, in pairs, irregular clusters and short chains (Fig. 2b). After transfer to fresh medium coccoid cells grew out to give rise to irregular slightly curved rods. Cells were heterogeneous in their sizes. Rods varied from 1.0 up to 3.1 µm in length and had a width of about 0.5–0.8 µm. The diameters of the cocci were 0.7–1.0 µm. The heterogeneity of the cells was additionally demonstrated by scanning electron microscopy (Fig. 3). Spore formation and motile cells were not observed.

The cells of strain HKI 0122^T stained Gram-positive and were not acid-fast according to Ziehl–Neelsen staining.

Strain HKI 0122^T grew well on complex organic media at 28 °C under aerobic and microaerophilic conditions. Growth was much reduced at 37 °C and did not occur at 42 °C nor under an anaerobic atmosphere, respectively. Restricted growth was observed on R agar with 2–4% (w/v) of NaCl. Growth was completely inhibited by the presence of 6% (w/v) of NaCl in the

Fig. 2. Micrographs of strain HKI 0122^T grown at 28 °C in liquid R medium for (a) 24 h or (b) 96 h. Bars, 10 µm.

Fig. 3. Scanning electron micrograph of cells from a 72-h-old culture of strain HKI 0122^T grown on R agar. Bar, 1 µm.

Table 1. Physiological properties of strain HKI 0122^T

–, Negative; +, positive; w, weakly positive.

Test	Result	Test	Result	Test	Result
Decomposition of:		Utilization of:		Growth in the presence of:	
Adenine	–	Acetate	–	2% NaCl	+
Casein	+	Aconitate	–	4% NaCl	+
Esculin	+	Benzoate	–	6% NaCl	–
Gelatin	+	Citrate	–	Growth at:	
Hippurate	–	Formate	–	28 °C	+
Hypoxanthine	–	Malate	–	37 °C	w
Potato starch	w	Succinate	–	42 °C	–
Tween 80	–	DL-Tartrate	–	Antibiotic susceptibility:	
Tyrosine	–	Enzyme assay (API ZYM)		Ampicillin, 10 µg	+
Urea	–	Phosphatase alkaline	+	Chloramphenicol, 30 µg	+
Xanthine	–	Esterase (C 4)	+	Ciprofloxacin, 5 µg	–
Acid produced from:		Esterase lipase (C 8)	+	Erythromycin, 15 µg	+
L-Arabinose	+	Lipase (C 14)	–	Gentamicin, 10 µg	–
Lactose	–	Leucine arylamidase	+	Kanamycin, 30 µg	–
D-Cellobiose	+	Valine arylamidase	–	Lincomycin, 2 µg	–
Dextrin	+	Cystine arylamidase	+	Neomycin, 30 µg	+
D-Fructose	+	Trypsin	–	Nitrofurantoin, 300 µg	–
D-Galactose	+	Chymotrypsin	–	Oxacillin, 5 µg	–
D-Glucitol	–	Phosphatase acid	+	Oxytetracycline, 30 µg	+
D-Glucose	+	Naphthol-AS-BI-phosphohydrolase	+	Penicillin, 2 IU	+
Glycerol	+	α-Galactosidase	+	Polymyxin B, 300 IU	w
Inulin	+	β-Galactosidase	+	Rifampin, 2 µg	+
Maltose	+	β-Glucuronidase	–	Streptomycin, 10 µg	–
D-Mannitol	–	α-Glucosidase	+	Sulfonamide, 300 µg	–
D-Mannose	+	β-Glucosidase	–	Nitrate reduction	+
D-Raffinose	+	N-Acetyl-β-glucosamidase	+	Production of H ₂ S	+
L-Rhamnose	+	α-Mannosidase	+	Catalase reaction	+
D-Ribose	+	α-Fucosidase	+	Methyl red test	–
Salicin	+			Voges-Proskauer test	–
Sucrose	+			Oxidase test	–
Potato starch	+			Indole test	–
Trehalose	+				
D-Xylose	+				

agar. Long-term storage was performed in the vapour phase of liquid nitrogen by adding 5% (w/v) of DMSO to the culture broth.

The physiological properties of strain HKI 0122^T are listed in Table 1.

Cell wall analysis

The peptidoglycan of strain HKI 0122^T contained Ala, Glu and Lys in a molar ratio of 2:1:2:2:1:0. Dinitrophenylated Glu was detected in hydrolysates of cell wall preparations treated with 1-fluoro-2,4-dinitrobenzene. Enantiomeric analysis of cell wall amino acids resulted in detection of D-Ala, L-Ala, D-

Glu, L-Glu and L-Lys. From these results and from two-dimensional thin-layer chromatographic peptide patterns of partial hydrolysates of cell walls (data not shown), it was concluded that strain HKI 0122^T contains a peptidoglycan of type A4α (Schleifer & Kandler, 1972) with an L-Lys ← L-Glu interpeptide bridge [murein type A11.54 (DSMZ, 1998)]. The acyl type was acetyl. The whole-cell sugars were glucose, mannose and galactose.

Lipid analysis

The polar lipid patterns consisted of phosphatidyl-inositol, diphosphatidylglycerol and three unknown phospholipids. The cellular fatty acid profiles were of

iso and anteiso branched type and were distinctly dominated by the occurrence of 13-methyl and 12-methyl tetradecanoic acids. The percentages of fatty acid composition of strain HKI 0122^T are as follows: i-C_{14:0}, 0.9; C_{14:0}, 1.9; i-C_{15:0}, 43.7; ai-C_{15:0}, 34.6; C_{15:0}, 0.9; i-C_{16:0}, 2.3; C_{16:0}, 6.8; i-C_{17:0}, 3.1; ai-C_{17:0}, 4.9; C_{18:1}, 0.9 (note: C_{16:0}, hexadecanoic acid; C_{18:1}, octadecenoic acid; i-C_{15:0}, 13-methyl tetradecanoic acid; ai-C_{17:0}, 14-methyl hexadecanoic acid).

The major menaquinone was MK-8(H₄). MK-8(H₂), MK-8 and MK-9(H₄) occurred in minor amounts. Mycolic acids were not present.

DNA base composition

Strain HKI 0122^T had a DNA G+C content of 71 mol %.

16S rDNA analyses

The almost complete 16S rDNA sequence, consisting of 1485 nucleotides (96.3% of the *Escherichia coli* sequence) was determined for strain HKI 0122^T. A 16S rDNA stretch of 530 nucleotides determined for strain HKI 0132 which was isolated from the same cave from which strain HKI 0122^T was isolated, showed 100% sequence similarity to the homologous region of the 16S rDNA of the latter strain. Comparison of the sequence of strain HKI 0122^T with those of the members of the order *Actinomycetales* revealed that this strain is a phylogenetic member of the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997), with which strain HKI 0122^T shared between 90% (with *Brevibacterium linens*) and 95% (with *Promicromonospora enterophila*) similarity. The sequence of strain HKI 0122^T possesses all of the signature nucleotides described to define members of this suborder (Stackebrandt *et al.*, 1997). Similarity values determined with sequences of members of the various families within this suborder ranged from 91.7 to 93.1 (*Dermatophilaceae*), 91.7 to 93.6 (*Microbacteriaceae*), 91.7 to 94.2 (*Micrococcaceae*) and 92.9 to 95.0 (*Cellulomonadaceae*). In this connection it should be noted, that the nucleotide pair U-U given for the position 598–640 for members of the family *Micrococcaceae* is not a signature but an error and should be corrected to U-G (Stackebrandt *et al.*, 1997).

The lack of high similarity values is consistent with the finding that strain HKI 0122^T shares only between 9 and 59% of signature nucleotides that phylogenetically define families within the suborder *Micrococcineae*. The highest values were with members of the families *Cellulomonadaceae* (59%) and *Intrasporangiaceae* (56%), indicating that the isolate HKI 0122^T does not belong to any of the genera contained in these families. Phylogenetic analysis of strain HKI 0122^T and the chemotaxonomically similar species *Demetria terrigena* DSM 11295^T and *Bogoriella caseilytica* DSM 11294^T revealed 91.6 and 93.0% 16S rDNA sequence similarities, respectively.

DISCUSSION

Lysine in position 3 of the peptide subunit of the peptidoglycan is widely distributed among actinomycetes representing distinct phylogenetic groups. The peptidoglycan type A11.54 (DSMZ, 1998) with the interpeptide bridge L-Lys ← L-Glu is only described for *Arthrobacter sulfureus* (Stackebrandt *et al.*, 1983). Although it was found that strain HKI 0122^T has the same structure of the interpeptide bridge and an *Arthrobacter*-like cell and colony morphology there were pronounced differences in the G+C content of the DNA, the menaquinone type, the composition of phospholipids and the fatty acid profile between these two taxa (Table 2). These differences justify the separation of strain HKI 0122^T from *A. sulfureus* at the genus level which is in agreement with the phylogenetic positions of strain HKI 0122^T and the *Arthrobacter* cluster (Fig. 1). Assessment of the phylogenetic position of strain HKI 0122^T within the radiation of a varying selection of reference organisms, using the formula of De Soete (Maidak *et al.*, 1997) the neighbour-joining (NJ) method and the maximum-parsimony method (Felsenstein, 1993), consistently showed this strain to branch outside the radiation of any of the defined families of the suborder *Micrococcineae*. Differences among the phylogenetic trees are seen in the branching points of deeply rooting lineages without, however, changing the genus position of the genera within the individual families. As noted by Stackebrandt *et al.* (1997) almost none of the branching points among actinobacterial families is supported by high bootstrap values; this phenomenon extends even to some intrafamily relationships when the radiation of lineages occurs within a short evolutionary time span (Stackebrandt *et al.*, 1997). The branching point of strain HKI 0122^T is depicted in the distance matrix dendrogram (Fig. 1), generated by the formula of De Soete (1983).

Strain HKI 0122^T is unrelated to the genera *Bogoriella* (Groth *et al.*, 1997a) and *Demetria* (Groth *et al.*, 1997b) with which it has in common lysine as the diagnostic diamino acid of the peptidoglycan and menaquinones of the MK-8(H₄) type. This separateness is supported by morphological, physiological and chemotaxonomic characteristics such as the composition of phospholipids and fatty acids (Table 1).

Two unnamed isolates from a medieval wall painting are a further example for the common occurrence of lysine and the menaquinone type MK-8(H₄) (Altenburger *et al.*, 1996). These two strains are similar to isolate HKI 0122^T in morphological and some chemotaxonomic features. However, their phylogenetic position, which was calculated on the basis of 900 bp of the 16S rDNA, reveals the highest degree of similarity to *Rathayibacter rathayi* (94.9%), family *Microbacteriaceae*. Therefore a close relationship between these isolates and strain HKI 0122^T is excluded.

Neither the genotypic nor phenotypic characteristics

Table 2. Differential characteristics of strain HKI 0122^T and selected taxa with murein type A4 α

Abbreviations: L-Ala, L-alanine; D-Asp, D-asparagine; L-Glu, L-glutamic acid; L-Lys, L-lysine; L-Ser, L-serine; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid; A, anteiso methyl-branched; I, iso methyl-branched; S, straight-chain saturated; U, monounsaturated.

Taxon	Cell morphology	G + C content (mol %)	Interpeptide bridge	Major menaquinone(s)	Polar lipids	Fatty acid types
HKI 0122 ^T	Rod-coccus cycle, non-sporulating	71	L-Lys $\leftarrow$ L-Glu	MK-8(H ₄)	PI, DPG, 3 PL	S, A, I
<i>Bogoriella</i> *	Irregular rods, coccoid, non-sporulating	70	L-Lys $\leftarrow$ L-Ala $\leftarrow$ L-Ala $\leftarrow$ L-Glu	MK-8(H ₄)	PG, DPG, PI, 1 PL	A, I
<i>Demetria</i> †	Coccoid, short rods, non-sporulating	66	L-Lys $\leftarrow$ L-Ser $\leftarrow$ D-Asp	MK-8(H ₄)	PI, PG, DPG, PE, 2 PL	S, U, A, I
<i>Arthrobacter sulfureus</i> ‡	Rod-coccus cycle, rods motile or non-motile, non-sporulating	64–66	L-Lys $\leftarrow$ L-Glu	MK-9 or MK-9, MK-10	PG, DPG, 1 PL	S, U, A, I

* Data from Groth *et al.* (1997a).

† Data from Groth *et al.* (1997b).

‡ Data from Stackebrandt *et al.* (1983).

of strain HKI 0122^T support an affiliation to a currently described genus or a family within the order *Actinomycetales*. Therefore we propose to classify strain HKI 0122^T in a new genus and species for which the name *Beutenbergia cavernae* gen. nov., sp. nov. is proposed. At this time we do not want to place the new genus in a new family, as the morphological and chemotaxonomic diversity, as well as the 16S rDNA signature nucleotides of single species cannot define the phylogenetic depth and width of a higher taxon.

Description of *Beutenbergia* gen. nov.

Beutenbergia (Beu'ten.berg.ia. -ia suffix to denote a locality; M.L. fem. n. *Beutenbergia* referring to Beutenberg, the geographical location of the institute in which the soil sample was studied).

Cells are irregular rods and cocci occurring singly, in pairs, short chains or clusters and exhibit a rod-coccus growth cycle. Cell sizes in rods vary from 1.0 to 3.1 μ m in length. The diameters of the cocci are 0.7–1.0 μ m. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. The peptidoglycan type is A4 α with L-Lys $\leftarrow$ L-Glu as interpeptide bridge. The acyl type is acetyl. The major menaquinone is MK-8(H₄). The major fatty acids are 13-methyl and 12-methyl tetradecanoic acids. The polar lipids are phosphatidylinositol, diphosphatidylglycerol and three unknown phospholipids. The G+C content of the DNA is 71 mol%. Phylogenetically, the genus is a member of the suborder *Micrococcineae*, order *Actinomycetales*. The type species is *Beutenbergia cavernae*.

Description of *Beutenbergia cavernae* sp. nov.

Beutenbergia cavernae (ca.ver'nae. L. masc. n. *cavus cave*; M.L. gen. fem. n. *cavernae* of a cave, referring to the habitat of the organism).

Cells are irregular rods and cocci occurring singly, in pairs, short chains or clusters and exhibit a rod-coccus growth cycle. Cell sizes in rods vary from 1.0 to 3.1 μ m in length. The diameters of the cocci are 0.7–1.0 μ m. Gram-positive, not acid-fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. Colonies are 0.7–1.8 mm in diameter, cream to bright yellow coloured, circular, convex. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, inulin, maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, sucrose, starch, trehalose and D-xylose. No acid production from D-glucitol, lactose or D-mannitol. Acetate, aconitate, benzoate, citrate, formate, malate, succinate and DL-tartrate are not utilized. Nitrate is reduced to nitrite, H₂S is produced, indole is not produced. Methyl red and Voges-Proskauer reactions are negative. Casein, aesculin, gelatin and potato starch are decomposed; adenine, hippurate, hypoxanthine, xanthine, Tween 80 and tyrosine are not decomposed. Urease activity is negative. NaCl in combination with R medium is well tolerated up to a concentration of 4% (w/v). Good growth occurs at 28 °C, weak growth at 37 °C, no growth at 42 °C. Cells are susceptible to ampicillin (10 μ g), chloramphenicol (30 μ g), erythromycin (15 μ g), neomycin (30 μ g), oxytetracycline (30 μ g), penicillin G (2 IU) and rifampin (2 μ g). Susceptibility to polymyxin B (300 IU) is weakly expressed, no susceptibility to ciprofloxacin (5 μ g), gentamicin (10 μ g), kanamycin (30 μ g), lincomycin (2 μ g), nitrofurantoin (300 μ g), oxacillin (5 μ g), streptomycin (10 μ g) and sulfonamide. Tested by the API ZYM enzyme assay strain HKI 0122^T is positive for phosphatase alkaline, esterase (C 4), esterase lipase (C 8), leucine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, and negative for lipase (C 14), valine arylamidase, trypsin,

chymotrypsin, β -glucuronidase and β -glucosidase. The peptidoglycan is of the A4 α type with an L-Lys $\leftarrow$ L-Glu interpeptide bridge. The acyl type is acetyl. The fatty acid pattern is of the iso and anteiso branched type. The major menaquinone is MK-8(H₄). The polar lipids are phosphatidylinositol, diphosphatidylglycerol and three unknown phospholipids. The G+C content of the DNA is 71 mol%. Mycolic acids are absent. The habitat is soil. The type strain is HKI 0122^T which has been deposited in the DSMZ as DSM 12333^T.

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